

any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Claims:

Please cancel claims 4, 5, 18, 19, 25, 26, 110, and 111 without prejudice to or disclaimer of the subject matter therein.

Please replace pending claims 1, 3, 15, 17, 22, 24, 106, 107, 109, and 158 with new claims 1, 3, 15, 17, 22, 24, 106, 107, 109, and 158.

1. (Twice Amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

- (a) obtaining a mammalian cell to be cultivated in suspension; and
- (b) contacting said cell with a serum-free, chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.
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3. (Amended) The method of claim 2, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

15. (Twice Amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

- E3
- (a) obtaining a mammalian cell to be cultivated in suspension; and
 - (b) contacting said cell with a chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca⁺⁺-pantothenate, folic acid, *D*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B₁₂, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, NaHCO₃, Na₂HPO₄, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

E4 17. (Amended) The method of claim 16, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

E5 22. (Twice Amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

- E⁵
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- (a) obtaining a mammalian cell to be cultivated in suspension; and
 - (b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.
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E⁶

24. (Amended) The method of claim 23, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

106. (Thrice Amended) A method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, said method comprising:

- E⁷
- (a) contacting said cells with a eukaryotic cell culture medium comprising a Fe^{2+} chelate and/or a Fe^{3+} chelate, and a Zn^{2+} salt, wherein said Fe^{2+} chelate, if present, said Fe^{3+} chelate, if present, and said Zn^{2+} salt are present in an amount which supports the growth of mammalian cells in culture, said medium further comprising a polyanionic or polycationic compound, and wherein said medium is capable of supporting the growth of mammalian cells in suspension culture and/or the expression of recombinant protein;

wherein said medium does not contain insulin or dextran sulfate; and

- (b) cultivating said mammalian cells under conditions suitable to support the growth of said cells and/or the expression of said recombinant protein.
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E⁸ 107. (Amended) The method according to claim 106, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

E⁹ 109. (Amended) The method according to claim 108, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

(158) (Amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

- E¹⁰
- (a) obtaining a mammalian cell to be cultivated in suspension; and
 - (b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

Please add the following new claims:

161. (New) A method of cultivating 293 cells in suspension *in vitro*, comprising:

- E¹¹
- (a) obtaining 293 cells to be cultivated in suspension; and
 - (b) contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension.

162. (New) The method of claim 161, wherein the medium further comprises at least one polyanionic or polycationic compound.

163. (New) The method of claim 162, wherein the polyanionic compound is a polysulfonated compound or a polysulfated compound.

164. (New) The method of claim 163, wherein the polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

165. (New) The method of claim 162, wherein the polysulfonated or polysulfated compound is dextran sulfate.

166. (New) The method of claim 165, wherein the dextran sulfate has an average molecular weight of about 5,000 daltons.

167. (New) The method of claim 162, wherein the medium is protein-free.

Sub F2 168. (New) The method of claim 162, wherein the medium further comprises one or more ingredients selected from the group of ingredients consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, one or more transferrin substitutes, insulin, and one or more insulin substitutes.

169. (New) The method of claim 168, wherein the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

170. (New) The method of claim 169, wherein the one or more plant peptides are one or more rice peptides or one or more soy peptides.

171. (New) The method of claim 168, wherein the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

172. (New) The method of claim 168, wherein the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca⁺⁺-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B₁₂.

173. (New) The method of claim 168, wherein the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, Fe(NO₃)₃, KCl, one or more magnesium salts, one or more manganese salts, NaCl, NaHCO₃, Na₂HPO₄, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

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174. (New) The method of claim 165, wherein the dextran sulfate is present in the
medium in an amount effective to substantially prevent clumping.

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